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EDITORIAL

Tight junctions: Guardians of the paracellular pathway

The contribution of tight junctions to the establishment and maintenance of epithelial cell polarity has long been recognized. Many studies on tight junctions have been performed on renal epithelial cells *in vitro*, but much less is known about their *in vivo* regulation and function. Early freeze-fracture work on the kidney demonstrated that tight junction structure is variable along the urinary tubule, and that the complexity of the tight junction could be roughly equated with the paracellular permeability (and electrical resistance) of the different tubule segments [1]. The study by Gonzalez-Mariscal et al in this issue of *Kidney International* [2] takes advantage of advances in our understanding of the molecular components of the tight junction to re-examine the relationship between junctional complexity, specific protein expression and transepithelial resistance in isolated tubule segments. They conclude that the distribution of the tight junction-associated proteins ZO-1, ZO-2 and occludin parallel junctional complexity, and that all of these proteins are expressed in significantly greater amounts in distal segments, including the collecting duct (a high-resistance, or "tight" epithelium), than in the proximal tubule (a low-resistance, or "leaky" epithelium). Thus, we have moved on from a purely morphological description of tight junctions in the kidney to a more detailed description of some of the protein components that are the building blocks of tight junctions along the urinary tubule.

When examined by freeze-fracture electron microscopy, tight junctions appear as linear strands or fibrils that represent the region of close apposition between plasma membranes from adjacent cells [1]. There was great controversy over the molecular nature of these strands, which was not settled by the discovery of the first tight junction-associated protein ZO-1 [3], because this protein turned out to be entirely cytoplasmic, with no transmembrane domain. Thus, the discovery of a membrane-spanning protein occludin, and the demonstration that it was associated with the typical fibrils seen in freeze fracture, was a significant advance in the field [3]. Surprisingly, however, occludin-deficient embryonic stem cells still formed morphologically "normal" tight junctions, and only when yet another family of membrane-spanning tight junction proteins was found, the claudins, did the picture become clearer [4]. It is now apparent that the tight junction consists of several peripheral membrane proteins (including ZO-1, ZO-2 and ZO-3) and at least two integral membrane proteins (occludin and the claudins) that contribute to the formation of the morphologically-detectable entity. The peripheral proteins subserve a number of functions, including regulating junctional assembly and interaction of the junctional complex with the actin cytoskeleton. The "ZO" proteins are membrane-associated guanylate kinases (MAGUKs) that modulate tight junction assembly and disassembly by a series of complex and poorly understood interactions with a number of other signaling elements, including small and large molecular weight GTP-binding proteins and protein kinases [3].

An understanding of the multitude of factors and signaling pathways that regulate tight junction assembly and disassembly is important for a number of reasons. First, without tight junctions, an epithelial barrier cannot be formed and vectorial transepithelial transport processes could not occur. Clearly, without the ability to move fluid, electrolytes and other molecules in a selective and directed fashion, the various epithelia that comprise the renal tubule (as well as epithelia in general) could not function. Indeed, such a breakdown in barrier function occurs after renal ischemia, when cell polarity is partially lost and is only gradually restored during the post-ischemic regeneration of a functional epithelium [5]. Loss of cell polarity also occurs in polycystic kidney disease, and inappropriate targeting of some transporters, including the Na,K-ATPase, may be at least partially responsible for the cystic phenotype. Interestingly, one of the gene products that is mutated in many cases of autosomal dominant polycystic kidney disease (ADPKD), polycystin 1, has been reported to form a complex with components of the adherens junctions in epithelial cells [6], and we have found that polycystin 1 co-localizes with ZO-1 in renal proximal tubule epithelial cells in situ (abstract; Brown et al, *J Am Soc Nephrol* 9:371, 1998). Thus, a role for polycystin 1 in the generation and/or maintenance of the polarized epithelial cell phenotype can be envisaged.

However, in addition to a role in the barrier function of epithelia, it has long been suspected that tight junctions may be selectively permeable to some ions, because the permselectivity of the paracellular pathway is variable in different segments of the renal tubule. It was, therefore, a major breakthrough when the first "extracel-

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lular ion channel" was reported recently by Simon et al [7]. Mutations in a human gene encoding a protein called paracellin-1 were found to be responsible for a renal magnesium wasting disease. Remarkably, paracellin-1 was found to be homologous to the claudin family of tight junction proteins. This discovery provides a plausible explanation for the finding that different members of the claudin family of proteins are expressed in different epithelia. Claudin 16 (or paracellin-1) is expressed in the thick ascending limb of Henle where it appears to function as a magnesium channel in the paracellular pathway. It is certain that specific ion-selective properties of other claudins, also expressed in specific epithelia, will emerge in the coming months and years.

Both occludin and the claudin family are also potential targets for microbial toxins and other proteins that result in epithelial pathologies. For example, the enterotoxin from Clostridium perfringens (CPE) binds to claudins 3 and 4 and causes a disintegration of the tight junction barrier in MDCK cells [8]. Tight junctions between lung epithelial cells are disrupted by a cysteine protease (Der p1) found in house dust mite feces, and it has been suggested that this is an initial step in the development of asthma in response to a variety of allergens [9]. There are many other examples of the pathophysiological effects associated with loss of tight junction integrity, including hepatic bile duct tight junctions perturbation in cholestasis [10]. Thus, it is not surprising that considerable effort is devoted to unraveling the multitude of interactions that contribute towards the establishment, development and modulation of a functional epithelial barrer. Mapping the distribution of the contributing proteins, as described by Gonzalez-Mariscal et al, is an initial step on the longer road that will hopefully allow us to understand how renal epithelia are constructed, how they allow some molecules but not others to traverse the paracellular pathway, and why the epithelial barrier fails during a variety of disease processes [2]. The ultimate aim is to develop therapeutic interventions that will either reduce the initial extent of epithelial damage after a specific insult such as ischemia, or that will allow epithelia to be quickly restored to a functional state before serious and irreversible progression towards renal failure occurs.

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